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Comparison of Thermoregulatory Devices Used during Anesthesia of C57BL/6 Mice and Correlations between Body Temperature and Physiologic Parameters

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General anesthesia affects several body systems, including thermoregulation. Decreased body temperature during anesthesia has potential negative effects, including delayed recovery to consciousness. Thermoregulatory support devices are used to maintain temperature in anesthetized rodents. We analyzed 2 novel thermoregulatory devices, thermogenic gel packs and reflective foils, to compare their effectiveness in maintaining temperatures with that of a standard circulating-warm–water blanket (CWWB) in C57BL/6 mice. Mice were grouped randomly: control (no thermal support), reflective foil, gel pack, gel pack plus reflective foil, CWWB on medium setting, CWWB on high setting, and CWWB on high setting plus reflective foil. Mice were anesthetized with isoflurane for 30 min, and temperature and heart and respiratory rates were monitored. Results indicated that the temperatures of mice with reflective foil only (start temperature, 36.2 ± 0.38 °C; end temperature, 28.8 ± 0.78 °C) did not differ significantly from those of control mice; however, the inclusion of foil heightened thermogenic properties when combined with other devices. Thermogenic gel packs and CWWB on high setting, both with and without reflective foil, caused significant temperature increases (that is, 1.6 °C to 4.4 °C) in mice. CWWB on medium setting (blanket temperature, 37.5 °C) maintained mice at temperatures within 1 °C of the 36.1 °C baseline. Strong correlations existed between temperature, heart and respiratory rates, and recovery time to consciousness. This information provides guidance regarding the use of thermoregulatory devices in anesthetized rodents and demonstrates the effect of maintaining a consistent core temperature on physiologic parameters.

Abbreviation: CWWB, circulating warm water blanket.

General anesthesia is administered frequently to biomedical research animals and causes physiologic alterations that affect many different body systems. Anesthesia inhibits several regulatory mechanisms of the body, including those that control the body's ability to maintain normothermia.[22,26,27] Therefore, appropriate thermoregulation is a critical element of safe anesthesia. In humans, general anesthesia inhibits the sympathetic nervous system, resulting in decreases in the vasoconstriction threshold, redistribution of core heat to peripheral limbs, a reduction in the ability to perform nonshivering thermogenesis, and impairment in temperature regulation by inhibition of the afferent transmission of thermal information.[26,27] Thermoregulation is complicated further in mice due to the high surface-area–to-mass ratio in this species, which predisposes them to hypothermia by heat loss.[25] In addition, flow of cold inhalant gasses, open incision sites, and fluid irrigation can lead to heat loss by convection and conduction during anesthetic and surgical procedures. The overall combination of these factors results in a drop in body temperature and potentially detrimental effects of hypothermia during and after anesthetic procedures.[6,28,35] Decreased temperature during anesthesia has been shown to increase rates of postoperative infections, coagulopathies, and cardiac arrhythmias in humans.[5,16,24] Because of the potentially deleterious effects of decreased temperature, thermal support should be provided to maintain a consistent body temperature during anesthetic procedures in mice and other small rodents.

Several methods have been proposed to prevent decreases of body temperature in small rodents during anesthesia or related experimental procedures. Notably, circulating warm water blankets (CWWB) and a microwaveable pad were effective at controlling body temperature of male CF1 mice maintained under isoflurane anesthesia.[33] These thermogenic items offered a refinement to standard practices, and potentially a safer method of support to rodents during anesthesia, as compared with electric heating blankets that can have local heating variability and may cause hyperthermia and focal burns to patients.[1,3,17] Additional novel items now are available to the laboratory animal community that also may provide thermal support for anesthetized rodents. These devices include reflective foil materials, which retain heat generated by the animal, and gel packets, which generate heat in a controlled fashion from chemical processes; descriptions of the relative thermogenesis are available from the device manufacturers.

Reflective foil (for example, Space Drape Pouch, Space Drapes, Manchester, MD) was designed to address several heat-loss mechanisms. The proposed method of action is due to reflection of any heat that naturally radiates from the animal and which then will be redirected back to the animal. When used in accordance with the manufacturer's instructions, the animal is surrounded by the reflective foil such that there is a

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uniform return of heat, as compared with traditional drapes where heat is supplemented primarily at the point of contact with the animal. The manufacturer states that the reflective foil helps counteract the loss of heat by convection and conduction through provision of a barrier between the rodent and the room environment, effectively limiting the amount of cold air and cooler surfaces (that is, benchtop) that come into contact with the animal. If a supplemental heat source is provided with the foil, the additional heat plus the insulation of the foil may be a more effective way to preserve the body temperature of the animal. Another novel device, thermal gel packets, provides a source of heat to keep the animal warm. The gel packet we analyzed (Space Gels, Space Drapes) is easy to transport and reuse; in addition, the packet is activated through initiation of a sustained chemical exothermic reaction of the contents.

We proposed to evaluate the effectiveness of the reflective foil material and thermal gel packets, alone and in combination, compared with a standard CWWB. Our study objectives were 3-fold: 1) to determine the efficacy of the novel devices in preventing hypothermia during anesthesia, given our hypothesis that both devices would prevent hypothermia and could effectively be combined with other methods for heightened efficacy; 2) to determine the correlation between body temperature and relevant physiologic parameters (for example, heart rate, respiratory rate, and time to regaining consciousness after anesthesia) and quantitatively identify the changes, given our hypothesis that these measures would be strongly correlated with body temperature; and 3) to determine an ideal surface temperature for thermal support devices for sustained maintenance of a consistent core temperature during anesthesia in mice.

Materials and Methods

Animals and experimental procedures. The University of Pennsylvania IACUC approved all of the procedures in this study. Female C57BL/6 mice (n = 48; age, 10 to 12 wk; Charles River Laboratories, Wilmington, MA) were used. All animals were housed at a 12:12-h light:dark cycle at a density of 5 mice per static polycarbonate microisolation cage (Max 75, Alternative Design, Siloam Springs, AR) on disposable bedding (diameter, 0.12-in.; Bed-O-Cobs, Animal Specialties and Provisions, Quakertown, PA). Wire lid food hoppers within cages were filled to capacity with rodent chow (Lab Diet 5010, Animal Specialties and Provisions) and maintained on water supplied by bottle.

A subset (n = 26) of the 48 mice was used to determine the average heart rate of unanesthetized mice. Heart rate was determined both at baseline and during anesthesia by using electrocardiography (ECGenie and eMouse 11 Analysis Software, Mouse Specifics, Quincy, MA). At least 1 wk before the procedure, individual IPTT300 transponders (BMDS, Seaford, DE) were injected subcutaneously into the dorsum of mice between the scapulae to transmit body temperature to a monitoring system (DAS-6007, BMDS). All microchip transponders were inserted as directed by the manufacturer, did not require additional calibration, and successfully provided data throughout the course of the experiments. The microchips are sensitive to within 0.5 C° and have a resolution of 0.1 C°. In one mouse, the chip migrated from the injection site, and a second chip had to be implanted the next day; no other chip failure was experienced.

Mice (n = 8 per group) were anesthetized for 30 min. Room temperatures were controlled by the HVAC system of the animal facility. Ambient environmental temperatures were recorded for every experiment and remained between 19.9 and 20.9 °C. Isoflurane (4% in O_2 at 1 L/min) was used for anesthesia by using a 2-L induction chamber (Vet Equip, Pleasanton, CA). Once mice were fully anesthetized, as determined by loss of the righting reflex (that is, mice could not consciously correct themselves to normal posture when placed in dorsal recumbency), isoflurane was reduced to a maintenance dose of 2% (1.5 minimum alveolar concentration) via a nose cone at a flow rate of 500 mL/min for 30 min. The time from induction of anesthesia to loss of the righting reflex, by using the described anesthetic regimen, was recorded for a second subset of mice (n = 20).

After induction of anesthesia, a temperature probe (RET3, Physitemp Instruments, Clifton, NJ) was placed intrarectally up to 19 mm (length of the probe) and held in place with white medical tape (Durapore, 3M, St Paul, MN) against the tail. The probe was connected to a thermometer (MicroTherma TW2-193, Thermoworks, Lindon, UT), which displayed the animal's rectal temperature. This thermometer is accurate within 0.2°C and captured data in real time. Rectal and chip temperatures were recorded (starting at time point 0) to ensure that body temperatures were captured appropriately; these thermometry methods have been shown to be interchangeable in a variety of laboratory species.[2,7,15,36] Electrocardiography leads then were applied, by using medical white tape, to the left forefoot and the middle of the ventral surface of the tail. After the time 0 reading, rectal temperature, subcutaneous microchip temperature, and heart and respiratory rates were recorded every 5 min for the full 30 min of anesthesia. At the conclusion of 30 min, isoflurane was discontinued, and mice received 100% oxygen supplementation at 500 mL/min while electrocardiography leads were disconnected. Once the leads were removed, mice were exposed to room air. Mice were monitored until they had recovered fully, as assessed by return of the righting reflex and normal ambulation. To gauge the effect of hypothermia on recovery from anesthesia, we measured 1) the time to first spontaneous movement after cessation of isoflurane and 2) the time to return of the righting reflex.

After the experiments, the mice were monitored for 5 d for any signs of skin pathology, potentially due to thermal injury, or abnormal behaviors. Each mouse in this study underwent no more than 2 anesthetic events and was rested 5 to 7 d between procedures.

Heating devices tested. The control group consisted of 8 mice that received no supplemental thermal support during anesthesia. Control mice were placed in ventral recumbency on a standard (18 in. × 24 in.) blue surgical Huck towel (Sklar Instruments, West Chester, PA) folded on itself so that they were not in contact with the steel surface of the benchtop (Figure 1 A). Another group of 8 mice were placed inside reflective foil (Space Drape Pouch, Space Drapes, Manchester, MD) so that they were completely surrounded by the reflective material. Electrocardiography leads and rectal probes were inserted via a small (1 cm) incision in the reflective foil (Figure 1 B) that minimally compromised that material. Three groups of 8 mice each were exposed to a CWWB (T/pump Classic, Gaymar Industries, Orchard Park, NY): one group at the medium setting (manufacturer's label of 38 °C), one group at the high setting (manufacturer's label of 42 °C), and one group with the CWWB on the high setting and inside reflective foil. These mice were placed in dorsal recumbency directly on the CWWB (Figure 1 C). Mice that were supplemented with the foil material and a heat source were placed directly on the heat source. The foil material was draped over the mice, allowing for reflection of heat from the primary device.

The final 2 groups of 8 mice were provided with thermal gel packets (Space Gels, Space Drapes), one group with the thermal gel packet alone, and the other with the thermal gel pack and reflective foil. The mice were placed on an insulating material (3/16-in.-thick bubble wrap) with the gel packet placed under

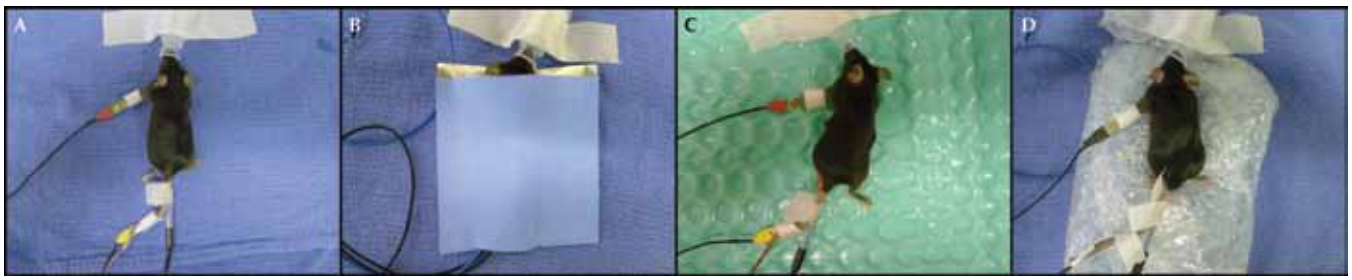

Figure 1. Experimental set-ups used for different testing groups. (A) Control group with no thermal support. (B) Mouse supported with a reflective foil pouch. (C) Mouse supported with CWWB. (D) Mouse supported with thermal gel pack encased in 4 layers of bubble wrap. All mice were monitored by using the front left limb and midventral tail for heart rate via electrocardiography, and temperatures were gathered by rectal thermometry while the mice maintained on isoflurane delivered via nosecone.

this material, according to the manufacturer's instructions. Four layers of bubble wrap were used between the mouse and gel packet to achieve surface temperatures consistently below 42 °C (Figure 1 D). The gel pack device was activated by snapping an internal metal disk while the gel pack was in the liquid state; the pack then was wrapped in the bubble wrap as described. The surface temperature of the area on which the mouse was resting was measured by using an infrared thermometer (Fluke, Everett, WA) at the beginning and end of the 30-min anesthetic event.

Monitoring of body temperatures to prevent hypothermia and hyperthermia. For all mice, rectal temperatures were not permitted to fall below 27.0 °C and were not permitted to rise above 41 °C, to avoid detrimental effects of hypothermia or hyperthermia as have been described in other species.[4,9,19,20,27,28,38] When the rectal temperature of a mouse reached 41 °C, the isoflurane anesthesia was discontinued immediately, and the mouse was cooled with a combination of flowing air via a fan and application of isopropyl alcohol to the tail and foot pads. For the experimental mice ($n = 3$) that met the criterion of a body temperature of 41 °C, data were not included in the analysis of times of recovery from anesthesia.

Statistical analysis. The relationship between rectal temperature and transponder temperatures was assessed by linear-regression analysis. Temperatures among the heating technique groups over the course of 30 min were compared by using 2-way ANOVA for repeated measures. Posthoc pairwise comparison Bonferroni corrections were performed when significant differences were found among heating device groups. Similar comparisons were made for the 2 measures of recovery rate. The correlations between rectal temperature and recovery time and between rectal temperature and heart rate were evaluated by using the Pearson correlation coefficient and associated P value. All statistical analyses were performed by using SAS version 9.2 (SAS Institute, Cary, NC). A 2-sided P value of less than 0.05 was considered to be statistically significant.

Results

Surface temperatures of warming devices. Mean surface temperatures for mice in the control group, foil-only group, and CWWB groups on the medium and high settings remained consistent throughout the 30-min anesthetic phase, with a variation of 0.2°C or less (Table 1). Surface temperatures of the CWWB were lower than manufacturer's labeled temperature on the device. The surface temperature of the thermogenic gel packs fell by the 30-min endpoint. The addition of the reflective foil to both the CWWB on high setting and the thermogenic gel pack increased surface temperatures from the starting surface temperature. Mean rectal temperatures of the mice at the start of each anesthetic exposure, regardless of thermogenic device to be tested, were essentially identical between groups.

Baseline physiologic effects. According to data from the subset of conscious mice ($n = 26$) used to determine baseline effects on heart rate and loss of righting reflex, the baseline heart rate (mean ± SEM) was 760 ± 21 bpm. Loss of righting reflex was measured in a second subset of mice ($n = 20$). The time until loss of the righting reflex during the induction of anesthesia with 4% isoflurane was 71.6 ± 8.3 s.

At the start of each testing phase, regardless of the device tested, there were no significant differences between the rectal temperatures of mice at time 0. However, significant effects on rectal temperature were noted over the 30 min of anesthesia and were specific to particular warming devices (Table 2). The control and foil-only groups showed similar temperature patterns, with significant ($P < 0.05$) differences between the groups at 5 and 10 min only. Mice in the control and foil-only groups had significant ($P < 0.05$) drops in body temperature at all time points compared with baseline, with temperatures that were significantly cooler than that at any other time point in any other group (Figure 2). The temperatures of the mice on the CWWB on the medium setting were significantly ($P < 0.05$) different from those of all other groups at all time points; this significance was attributed to the fact that this device provided consistent thermal support throughout the anesthetic event. Within the CWWB (medium) group, the only significant difference over time occurred between the starting temperature and that at the 30-min time point; yet this difference from beginning to end of the recording phase was the smallest of all devices assessed. The group supported with the gel pack and reflective foil were much warmer than were the mice in all other groups; these differences reached significance ($P < 0.05$) by the 10-min time point for the group with the CWWB on the high setting, by the 15-min time point for mice given the gel pack without reflective foil, and by the 20-min time point for the mice given the CWWB on high with reflective foil (Figure 2). In the group of mice supplemented with the gel pack and reflective foil, 3 of the 8 scheduled anesthetic procedures had to be terminated early when mice reached rectal temperatures of 41.0 °C. The gel-pack–only group did not differ significantly from the groups provided the CWWB on high setting with and without the reflective foil at any time point. Interestingly, at the 25- and 30-min time points, the rectal temperature of the mice with the CWWB on the high setting was significantly ($P < 0.05$) different from that of those having the CWWB on high setting and reflective foil.

Body temperature and physiologic parameters. We found that a strong correlation ($r = 0.98$, $P < 0.0001$) between temperatures acquired rectally and those obtained by subcutaneous microchips. Other studies report similar findings, which[2,7,15,36] we verified here in healthy laboratory mice monitored while anesthetized (data not shown). Body temperatures strongly correlated with

Table 1. Starting and final surface temperatures of all devices tested and starting rectal temperatures and weights of mice.

Device	Starting surface temperature (C°)	Final surface temperature (C°)	Starting rectal temperature (C°)	Weight of mice (g)
Control	22.8 ± 0.6	23.0 ± 0.5	36.2 ± 0.5	22.3 ± 0.31
Reflective foil	22.8 ± 0.6	22.6 ± 0.5	36.1 ± 0.2	22.6 ± 0.18
Gel pack	42.2 ± 0.6	41.5 ± 0.7	36.3 ± 0.4	21.9 ± 1.48
Gel pack and reflective foil	42.4 ± 0.4	43.4 ± 0.4	36.4 ± 0.3	21.9 ± 0.30
CWWB on medium setting	37.5 ± 0.3	37.5 ± 0.1	36.1 ± 0.4	23.6 ± 0.32
CWWB on high setting	40.8 ± 0.5	40.8 ± 0.4	36.4 ± 0.5	21.8 ± 0.25
CWWB on high setting and foil	41.1 ± 0.2	41.7 ± 0.4	36.5 ± 0.3	21.6 ± 0.18

Mice (n = 8 for each device tested) were placed in ventral recumbency on device surfaces during the 30 min of anesthesia. All values are presented as mean ± SE.

Table 2. Rectal temperatures (mean ± SE; n = 8 per group) of mice during anesthesia

Device group	Rectal temperature (C°)						
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
Control	36.2 ± 0.2	34.0 ± 0.3[a]	32.7 ± 0.4[a]	31.6 ± 0.4[a]	30.5 ± 0.4[a]	29.6 ± 0.4[a]	28.7 ± 0.4[a]
Reflective foil	36.1 ± 0.1	34.3 ± 0.2[a]	33.3 ± 0.4[a]	31.9 ± 0.3[a]	30.9 ± 0.2[a]	29.8 ± 0.2[a]	29.0 ± 0.2[a]
Gel pack	36.3 ± 0.1	37.2 ± 0.3[a]	37.8 ± 0.3[a]	38.2 ± 0.3[a]	38.3 ± 0.3[a]	38.5 ± 0.3[a]	38.6 ± 0.3[a]
Gel pack and reflective foil	36.4 ± 0.1	37.4 ± 0.1[a]	38.6 ± 0.2[a]	39.5 ± 0.1[a]	40.2 ± 0.1[a]	40.6 ± 0.1[a]	40.8 ± 0.1[a]
CWWB on medium setting	36.1 ± 0.1	35.9 ± 0.1	35.7 ± 0.1	35.6 ± 0.1	35.5 ± 0.1	35.4 ± 0.1	35.2 ± 0.1[a]
CWWB on high setting	36.4 ± 0.2	36.9 ± 0.2	37.3 ± 0.2[a]	37.7 ± 0.2[a]	37.9 ± 0.1[a]	37.9 ± 0.1[a]	38.0 ± 0.1[a]
CWWB on high setting with foil	36.5 ± 0.1	37.3 ± 0.2[a]	38.0 ± 0.3[a]	38.5 ± 0.3[a]	38.8 ± 0.3[a]	39.0 ± 0.2[a]	39.1 ± 0.2[a]

Temperatures were recorded every 5 min throughout the 30 min anesthetic event.
[a]Value significantly ($P < 0.05$) different from the 0-min (baseline) temperature.

both heart rate (r = 0.96, $P < 0.0001$) and respiratory rate (r = 0.88, $P < 0.0001$). The relationship between temperature and heart and respiratory rate transitioned at 36.1 °C (Figure 3) and 36.2 °C (Figure 4), respectively, with increases in both heart and respiratory rates linked with increases in body temperature.

Relationship between recovery times and body temperature. Significant differences were seen between various groups and recovery times to consciousness from anesthesia. The baseline rectal temperature (time point, 0 min; mean ± SEM) for all tests (n = 56) was 36.3 ± 0.4 °C. Mice whose body temperature dropped more than 2 °C from baseline had prolonged recovery times, and those that maintained temperatures at or above starting values had more rapid recovery times (Table 3). As would be predicted from the body temperatures measured at the 30-min time point, there were 2 distinct clusters of the experimental groups when examining recovery time. Between the 2 groups (controls and reflective foil only) in which anesthetized mice had the greatest loss of temperature from baseline values, there were no significant differences between time to first movement or restoration of the righting reflex. This finding again indicates that the reflective foil was essentially providing no thermal support, similar to conditions for control mice. Likewise, when the 5 other thermal support device combinations were compared, there were no differences between time to first movement and restoration of the righting reflex. The correlation coefficient for the relationship between body temperature and time until first movement was −0.80 ($P < 0.0001$) and between body temperature and restoration of the righting reflex was −0.85 ($P < 0.0001$); these data indicate that a decreased body temperature resulted in delayed recovery from anesthesia. After recovery, mice showed no overt adverse effects from exposure to anesthesia or thermogenic devices, and all appeared to be clinically normal. No further tests were performed to evaluate the effects of any body temperature variability that mice experienced.

Discussion

The current study had 3 main goals. One was to determine the surface temperature of thermal support devices that would maintain a constant body temperature in anesthetized mice throughout a procedure. Our studies were intended to produce information that would allow researchers to evaluate the ability of a particular heating device, by assessing its surface temperature, to maintain thermal support for mice under anesthesia. We found that a surface temperature of approximately 37.5 °C (achieved with the CWWB on the medium setting) kept murine body temperatures stabilized over the entire 30-min period of isoflurane inhalant anesthesia. We also evaluated the ability of 2 novel devices to provide thermal support to mice undergoing inhalant anesthesia and tested correlations between body temperature and heart and respiratory rates during anesthesia. Last, we evaluated the body temperatures of mice as they returned to consciousness (regained the righting reflex) after anesthesia.

Significant differences existed between the various warming devices and combinations tested in this study. The only method that did not provide supportive heat for anesthetized mice was the reflective foil when used alone. The body temperatures of the mice supplemented with the reflective foil device alone were not significantly different from those of the control mice, which had no heat source provided to them. Temperatures of mice in these groups dropped rapidly at all time points, with subsequent depression of heart and respiratory rates and a significantly prolonged recovery from anesthesia. This finding indicated that the reflective foil preserved very little of the body heat produced by the mice. The airflow from delivery of the anesthetic from the nose cone may have disrupted the direct reflection of the heat radiating from the mice, thus affecting the ability of the foil to preserve the animal's body heat. The foil device alone may provide improved thermogenesis when using an injectable anesthetic regimen for mice but further testing, beyond the scope

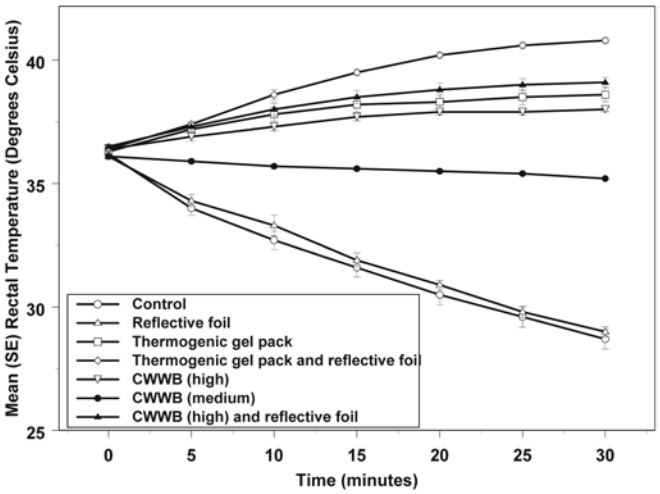

Figure 2. Mean (n = 8) rectal temperature of mice over time in each device group. Rectal temperatures were recorded every 5 min during anesthesia.

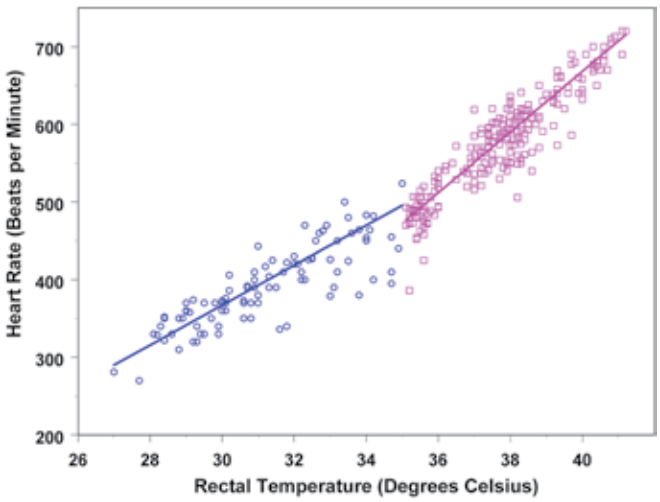

Figure 3. Scatterplot of rectal temperature compared with heart rate, with regression lines.

of our study of isoflurane anesthesia, is necessary to determine this outcome. When the reflective foil was combined with other thermogenic devices, we noted significant increases in rectal temperatures when compared with those from the heat source without reflective foil. These findings suggest that reflective foil may be more beneficial to animals when paired with an additional thermogenic device than when used alone.

The manufacturer of the thermogenic gel pack recommended using an insulating layer between the mice and gel pack to avoid placement of the animal directly on the heat source. Therefore, we applied 4 layers of bubble-wrap packing material, a lightweight, malleable, air-filled plastic material. The gel pack produced elevated surface temperatures, despite the layer of insulation, and there was a significant increase in body temperature, averaging 2.3 °C over the course of the 30-min experiment. When combined with the reflective foil, the gel pack produced extremely high body temperatures in mice, increasing the average body temperature of the mice by 4.2 °C. Recommendations based on larger species suggest that the surface temperature of a thermal support device should not exceed 42 °C.[8] In the current study, the starting temperatures of the gel pack with and without reflective foil were in this range; however, the surface temperature unexpectedly increased when the gel was combined with the

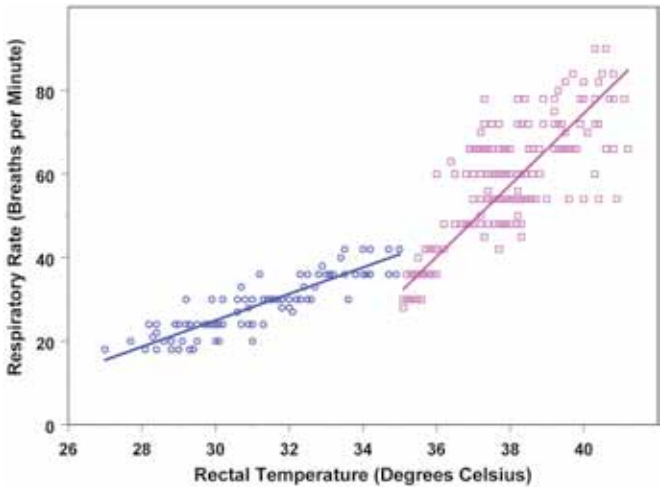

Figure 4. Scatterplot of rectal temperature compared with respiratory rate, with regression lines.

reflective foil. Although none of the mice in this study showed any skin lesions after recovery that might have been attributed to hyperthermia, we assert that the mice underwent a significant hyperthermic experience and therefore do not recommend the use of the gel–foil be for laboratory mice. Even modest elevations in body temperature have been shown to affect numerous functional aspects of biology (for example, immune and hepatic function) and should be avoided during anesthesia, just as hypothermia should similarly be avoided.[10,11,21]

The CWWB model used in this study had 3 built-in temperature settings, and the medium and high settings were used in this study. The CWWB on medium setting maintained a surface temperature of 37.5 °C and did a superior job at maintaining thermoneutrality in mice, with the mean rectal temperature dropping only 0.9 °C from time point 0 to the final time point at 30 min. We feel that this device, at the medium setting, provided a safe and effective means of thermal control, as confirmed in previous studies using this device.[29,33] The heart and respiratory rates remained nearly constant in this group. In light of these results, it should be noted that if normal physiologic body temperature is maintained during isoflurane anesthesia, changes in heart and respiratory rates could be used to determine changes in anesthetic depth of the mouse. Significant reductions in heart and respiratory rates during such an anesthetic event could be interpreted as the mouse transitioning into a deeper plane of anesthesia.

The CWWB on the high temperature setting caused a moderate (approximately 1.6 °C) elevation of rectal temperatures. As discussed earlier, any continued elevation in body temperature could be a concern due to unanticipated effects on the study resulting in confounding data. The use of multiple heat settings on the CWWB allows investigators to choose which would be most appropriate for the procedure being performed. The addition of the reflective foil to the CWWB on the high setting caused an increase in body temperature of 2.6 °C from baseline body temperature. Both of these warming methods, in addition to the gel packet alone, accomplished stabilization of body temperature after 15 min of anesthesia. This outcome was not unexpected, given that the surface temperature of these thermal devices remained fairly constant over 30 min, allowing the mice to reach a thermal equilibrium at approximately 1 to 2 °C below the surface temperature of the supporting device. Additional factors for consideration in future work are of potential strain-associated differences between the response to anesthesia and body temperature and that our experimental mice did not undergo a surgical procedure. Because

Table 3. Recovery time (mean ± SE) of mice after isoflurane anesthesia was discontinued

Device group	Recovery time (min)	
	Until first movement	Until restoration of righting reflex
Control	3.6 ± 0.5[a,b]	6.7 ± 0.6[d]
Reflective foil	3.6 ± 0.5[a]	6.5 ± 0.5[d]
Gel pack	1.3 ± 0.1[c]	2.2 ± 0.2[e]
Gel pack and reflective foil	1.2 ± 0.1[c]	2.9 ± 0.4[e]
CWWB on medium setting	2.2 ± 0.2[b,c]	3.5 ± 0.1[e]
CWWB on high setting	1.2 ± 0.1[c]	2.1 ± 0.2[e]
CWWB on high setting and reflective foil	1.0 ± 0.1[c]	2.2 ± 0.3[e]

All groups contained 8 mice, except the group that had the gel pack with foil group (n = 5). Values with different superscripted letters differed significantly ($P < 0.05$).

of heat loss from any necessary surgical incisions, a laparotomy or thoracotomy procedure may well require additional thermal support to maintain thermoneutrality, compared with that of the mice in our study.

The tested devices provided varying degrees of thermal support, thereby allowing investigation into the association between body temperature and the physiologic parameters of cardiac and respiration effects in isoflurane-anesthetized mice. We noted a marked drop in heart rate from 760 bpm in conscious mice to approximately 500 bpm at 5 min after the induction of isoflurane anesthesia; this phenomenon has occurred in previous studies.[12] Although cardiac depression from isoflurane is less severe than that of other anesthetic agents, this inhalant has been shown to have depressive effects on the sympathetic nervous system that result in cardiovascular changes.[13,30,40]

As body temperatures changed in our anesthetized mice, we noted similar changes in their heart and respiratory rates, with a strong positive correlation between these parameters. The normal physiologic response of mice to reduced body temperature is to increase activation of nonshivering thermogenesis and increase heart rate through the sympathetic nervous system.[14,31,32,39] Previous studies have shown that isoflurane can depress the sympathetic nervous system.[30] This effect may account for why these mice were unable to increase their heart rate in response to dropping body temperatures. Although our study was not intended to evaluate the mechanism of action that links core temperatures to cardiac and respiratory functions, we hypothesize that the animals' metabolic rate, which is strongly linked to their body temperature, may be driving the heart rate. In our study, a near doubling of the heart rate occurred with body temperatures of 30 °C and 40 °C (Figure 3). This finding is consistent with the Q10 effect, which predicts that with every 10 °C increase in temperature, the rate of enzymatic reactions, and subsequently metabolic rate, will double.[34] Within that same 10 °C temperature range, there is an even greater relative increase in respiratory rate, which shows an almost 3-fold increase. We hypothesize that this increase may be due to the mice's attempt to thermoregulate and dissipate excessive heat.

The scatter plots for heart and respiratory rates as a function of temperature appear to show 2 distinct slopes on each plot (Figures 3 and 4). These slopes intersected at 36.1 °C for heart rates and 36.2 °C for respiratory rates. These data indicate a physiologic shift at approximately 36.0 °C, suggesting that factors, in addition to metabolic rate, may be controlling heart and respiratory rates while mice are under inhalant anesthesia. Additional research into this phenomenon is necessary to further elucidate the underlying mechanisms.

Rapid and uncomplicated recovery is an important goal of any anesthetic experience. We noted that the body temperatures at the 30-min time point had a significant effect on the recovery time of mice. Those with diminished body temperatures required significantly more time to recover from the anesthetic exposure. The mean times until first movement and until restoration of the righting reflex after discontinuation of isoflurane anesthesia were 2- to 3- fold higher in the control and reflective foil groups compared with other groups. The delay in recovery associated with hypothermia has been well documented in other species.[18,23,37] On the basis of our observations, mice in the control and foil only groups had recoveries that appeared more physical in nature, with increased shivering, incoordination, and sporadic movements. These data and observations demonstrated the value of keeping mice warmed to best minimize adverse effects of recovery after even minimal anesthetic exposures.

We elected to use 2 methods of thermometry to collect body temperatures: rectal probes and subcutaneous transponder chips. Our studies and others have demonstrated very strong correlation between the 2 measures, although they fundamentally are measuring temperatures at different body sites.[2,7,15,36] The rectal body temperature is the 'gold standard' estimate of core body temperature, whereas the subcutaneous transponder may be perceived as a more likely recording of dermal temperature, which can be affected by local factors. However, with the knowledge that the methods are strongly correlated, there is a continued interest in using less-invasive and refined measures for assessing temperatures remotely (that is, by chips). Because these animals were healthy naïve mice, we decided to use rectal temperature values as the preferred measure because it did not unduly stress the study animals.

Hypothermia during anesthetic procedures in small rodents is an important complication, potentially compromising both animal health and data quality. To combat this effect, new devices for thermal support during anesthesia are routinely being developed and evaluated. The reflective foil alone provided minimal thermal support to mice during isoflurane anesthesia, but foil did augment the thermal support provided by other devices. The tested thermal gel packets also provided ample thermal support, but careful oversight and monitoring must be undertaken when using this device, because of the potential to cause marked hyperthermia. We recommend that the presence of adequate insulation between the mouse and gel pack is crucial for the safe use of this thermal device. We do not recommend using gel packs without carefully monitoring the surface temperature of the material used for insulation.

Among the options we evaluated, the CWWB on the medium temperature setting performed well, was reliable between animals, and was the most consistent in preserving temperature over time. We found that a surface temperature of 37.5 °C minimized changes in the mouse body temperature during the

30-min inhalant anesthetic exposure. In conclusion, the only device we unequivocally recommend currently is the CWWB on the medium setting, but careful manipulation and monitoring of the other devices tested, to keep them at surface temperatures close to 37.5 °C, may likely permit them to be used effectively in the laboratory animal setting.

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